Terms	Documents
(2 adj O adj methyl) same (amplify or amplification)	15

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☐ 1. Document ID: US 6291176 B1

L1: Entry 1 of 15 File: USPT Sep 18, 2001

DOCUMENT-IDENTIFIER: US 6291176 B1
TITLE: Identification of a DNA region potentially useful for the detection of mycobacterium kansasii

DEPR:
Suitable bases for preparing the oligonucleotide probes or amplification primers of the present invention may be selected from naturally occurring nucleotide bases such as adenine, cytosine, guanine, uracil, and thymine; and non-naturally occurring or "synthetic" nucleotide bases such as 8-oxo-guanine, 6-mercaptoguanine, 4-acetylcytidine, 5-(carboxyhydroxyethyl)uridine, 2'-O-methylcytidine, 5-carboxymethylamino-methyl-2-thioridine, 5-carboxymethylaminomethyluridine, dihydrouridine, 2'-O-methylpseudouridine, .beta., D-galactosylqueosine, 2'-O-methylguanosine, inosine, N6-isopentenyladenosine, 1-methyladenosine, 1-methylpseudouridine, 1-methylguanosine, 1-methylinosine, 2,2-dimethylguanosine, 2-methyladenosine, 2-methylguanosine, 3-methylcytidine, 5-methylcytidine, N6-methyladenosine, 7-methylguanosine, 5-methylaminomethyluridine, 5-methoxyaminomethyl-2-thiouridine, .beta.,D-mannosylqueosine, 5-methoxycarbonylmethyluridine, 5-methoxyuridine, 2-methylthio-N6-isopentenyladenosine, N-((9-.beta.-D-ribofuranosyl-2-methylthiopurine-6-yl)carbamoyl)threonine, N-((9-.beta.-D-ribofuranosylpurine-6-yl) N-methyl-carbamoyl)threonine, uridine-5-oxyacetic acid methylester, uridine-5-oxyacetic acid, wybutoxosine, pseudouridine, queosine, 2-thiocytidine, 5-methyl-2-thiouridine, 2-thiouridine, 2-thiouridine, 5-methylurdine, . N-((9-.beta.-D-ribofuranosylpurine-6-yl)carbamoyl)threonine, 2'-O-methyl-5-methyluridine, 2'-O-methylurdine, wybutosine, and 3-(3-amino-3-carboxypropyl)uridine. Any oligonucleotide backbone may be employed, including DNA, RNA (although RNA is less preferred than DNA), modified sugars such as carbocycles, and sugars containing 2' substitutions such as fluoro and methoxy. The oligonucleotides may be oligonucleotides wherein at least one, or all, of the internucleotide bridging phosphate residues are modified phosphates, such as methyl phosphonates, methyl phosphonothioates, phosphoromorpholidates, phosphoropiperazidates and phosphoramidates (for example, every other one of the intemucleotide bridging phosphate residues may be modified as described). The oligonucleotide may be a "peptide nucleic acid" such as described in P. Nielsen et al., Science 254, 1497-1500 (1991). The only requirement is that the oligonucleotide probe should possess a sequence at least a portion of which is capable of binding to a portion of the sequence of a target DNA molecule.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

☐ 2. Document ID: US 6268148 B1

L1: Entry 2 of 15 File: USPT Jul 31, 2001

DOCUMENT-IDENTIFIER: US 6268148 B1
TITLE: Detection of nucleic acid sequence differences using coupled ligase detection and polymerase chain reactions

DEPR:
FIG. 12 is a schematic drawing showing a solution to the allele specific LDR/PCR problem, utilizing an intermediate exonuclease digestion step. Allele-specific LDR/PCR can be achieved while significantly reducing background ligation independent (incorrect) target amplification. To do so, it is necessary to eliminate one or more of the components required for ligation independent PCR amplification, without removing the information content of the ligation product sequence. One solution is to use exonuclease in step 2 to digest unreacted LDR oligonucleotide probes from step 1. By blocking the end which is not ligated, for example the 3' end of the downstream oligonucleotide probe, one probe can be made substantially resistant to digestion, while the other is sensitive. Only the presence of full length ligation product sequence will prevent digestion of the upstream primer. Blocking groups include use of a thiophosphate group and/or use of 2-O-methyl ribose sugar groups in the backbone. Exonucleases include Exo I (3'-5'), Exo III (3'-5'), and Exo IV (both 5'-3' and 3'-5'), the later requiring blocking on both sides. One convenient way to block both probes is by using one long "padlock" probe (see M. Nilsson et. al., "Padlock Probes: Circularizing Oligonucleotides for Localized DNA Detection," Science 265: 2085-88 (1994), which is hereby incorporated by reference), although this is by no means required. An advantage of using exonucleases, for example a combination of Exo I (single strand specific) and Exo III (double strand specific), is the ability to destroy both target and one LDR probe, while leaving the ligation product sequences substantially undigested. By using an exonuclease treatment prior to PCR, in accordance with steps 3 and 4, either one or both oligonucleotide probes in each set are substantially reduced, and thus hybridization of the remaining oligonucleotide probes to the original target DNA (which is also substantially reduced by exonuclease treatment) and formation of a ligation product sequence which is a suitable substrate for PCR amplification by the oligonucleotide primer set is substantially reduced. In other words, formation of ligation independent labeled extension products is substantially reduced or eliminated.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

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☐ 3. Document ID: US 6130038 A

L1: Entry 3 of 15 File: USPT Oct 10, 2000

DOCUMENT-IDENTIFIER: US 6130038 A
TITLE: Method for amplifying target nucleic acids using modified primers

DEPR:
In another preferred embodiment, modified oligonucleotide primers are used in a nucleic acid amplification reaction in which a target nucleic acid is RNA. See, e.g., Kacian and Fultz, supra. The target may be the initially present nucleic acid in the sample, or may be an intermediate in the nucleic acid amplification reaction. In this embodiment, the use of preferred 2'-modified primers, such as oligonucleotides containing 2'-O-methyl nucleotides, permits their use at a higher hybridization temperature due to the relatively higher T.sub.m conferred to the hybrid, as compared to the deoxyoligonucleotide of the same sequence. Also, due to the preference of such 2'-modified oligonucleotides for RNA over DNA, competition for primer molecules by non-target DNA sequences in a test sample may also be reduced. Further, in applications wherein specific RNA sequences are sought to be detected amid a population of DNA molecules having the same (assuming U and T to be equivalent) nucleic acid sequence, the use of modified oligonucleotide primers having kinetic and equilibrium preferences for RNA permits the specific amplification of RNA over DNA in a sample.

DEPR:
Additionally, oligonucleotides may be modified to have hybridization kinetics and/or equilibrium preferences for a specific type of nucleic acid, such as RNA or DNA. As disclosed above, for example, 2'-O-methyl oligonucleotides preferentially hybridize with RNA over DNA. Thus, target capture oligonucleotides containing 2'-O-methyl nucleotides may be used to specifically capture RNA target nucleic acids, such as mRNA or rRNA, under hybridization conditions not promoting hybridization of the oligonucleotide to the genomic versions thereof. Likewise, 2'-O-methyl-modified amplification oligonucleotides and/or labeled probes can be designed, thereby targeting RNA over DNA for amplification and/or detection, as described above.

DEPR:
It is a further object of the present invention to provide kits including one or more oligonucleotides containing modified nucleotides which function to increase the rate of hybridization between the oligonucleotide and a target nucleic acid. Kits of the present invention could include any combination of probe, amplification, helper and sample processing oligonucleotides. In a preferred embodiment, the modified oligonucleotides of these kits would contain at least one cluster of about 4 2'-O-methyl modifications to the ribofuranosyl ring. Kits containing these modified oligonucleotides may be supplied for use in both diagnostic hybridization assays and amplification assays. Such kits may further include written instructions directing practitioners in the use of the modified oligonucleotides in either or both diagnostic hybridization assays or amplification assays.

Full | Title | Citation | Front | Review | Classification | Date | Reference   KWIC | Draw Desc | Image

☐ 4. Document ID: US 6117635 A
L1: Entry 4 of 15   File: USPT   Sep 12, 2000

DOCUMENT-IDENTIFIER: US 6117635 A
TITLE: Nucleic acid amplification oligonucleotides with molecular energy transfer labels and methods based thereon

DEPR:
A schematic illustration of gap triamplification, which consists of repeated elongation and ligation of the amplification product, is shown in FIG. 7. Blocker may be used at the same or higher concentration than the concentration of forward and reverse primers. Preferably, blocker is used at a 1.2 to 2-fold higher concentration than the concentration of forward and reverse primers. The primer complementary to the blocker preferably is modified to prevent strand displacement during amplification; in a preferred embodiment, this primer contains 2'-O-methyl at the position complementary to the 5' end of the blocker in order to prevent strand displacement.

DEPR:
Three oligodeoxynucleotides complementary to segments of human prostate specific antigen (PSA) DNA were synthesized (FIG. 12). Reverse primer contained a 2'-O-methyl moiety at a position complementary to the 5'-end of the blocker. This modification was essential for prevention of strand displacement during the amplification process (see Section 5.2.2.1) The blocker had biotin on its 3' end, in order to protect it from 3'-5' hydrolysis and from undesirable extension during amplification. During the synthesis of blocker and forward primer, the primary amino group was incorporated on the modified T-base (Amino-Modifier C6 dT) as described by Ju et al. (1995, Proc. Natl. Acad. Sci. USA 92:4347-4351). These modifications were used for subsequent incorporation of fluorescent dyes into designated positions of the oligonucleotides. Synthesized oligonucleotides were desalted and FAM (as a donor) and rhodamine (as an acceptor) were attached to a modified thymidine residue of the reverse primer and blocker, respectively, by the method published by Ju et al. (1995, Proc. Natl. Acad. Sci. USA 92:4347-4351). Labeled oligonucleotides were purified on a 15% denaturing polyacrylamide gel.

Full | Title | Citation | Front | Review | Classification | Date | Reference   KWIC | Draw Desc | Image

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☐ 5. Document ID: US 6100027 A

L1: Entry 5 of 15   File: USPT   Aug 8, 2000

DOCUMENT-IDENTIFIER: US 6100027 A
TITLE: Nucleic acid probes and amplification oligonucleotides for Neisseria species

BSPR:
By "oligonucleotide" is meant a single-stranded nucleotide polymer made of more than 2 nucleotide subunits covalently joined together. Preferably between 10 and 100 nucleotide units are present, most preferably between 12 and 50 nucleotides units are joined together. The sugar groups of the nucleotide subunits may be ribose, deoxyribose or modified derivatives thereof such as 2'-O-methyl ribose. The nucleotide subunits of an oligonucleotide may be joined by phosphodiester linkages, phosphorothioate linkages, methyl phosphonate linkages or by other rare or non-naturally-occurring linkages that do not prevent hybridization of the oligonucleotide. Furthermore, an oligonucleotide may have uncommon nucleotides or non-nucleotide moieties. An oligonucleotide as defined herein is a nucleic acid, preferably DNA, but may be RNA or have a combination of ribo- and deoxyribonucleotides covalently linked. Oligonucleotide probes and amplification oligonucleotides of a defined sequence may be produced by techniques known to those of ordinary skill in the art, such as by chemical or biochemical synthesis, and by in vitro or in vivo expression from recombinant nucleic acid molecules, e.g., bacterial or retroviral vectors. As intended by this disclosure, an oligonucleotide does not consist of wild-type chromosomal DNA or the in vivo transcription products thereof. One use of a probe is as a hybridization assay probe; probes may also be used as in vivo or in vitro therapeutic amplification oligomers or antisense agents to block or inhibit gene transcription, or translation in diseased, infected, or pathogenic cells.

Full | Title | Citation | Front | Review | Classification | Date | Reference | KWIC | Draw Desc | Image

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6. Document ID: US 6090552 A
L1: Entry 6 of 15 File: USPT Jul 18, 2000

DOCUMENT-IDENTIFIER: US 6090552 A
TITLE: Nucleic acid amplification oligonucleotides with molecular energy transfer labels and methods based thereon

DEPR:
A schematic illustration of gap triamplification, which consists of repeated elongation and ligation of the amplification product, is shown in FIG. 7. Blocker may be used at the same or higher concentration than the concentration of forward and reverse primers. Preferably, blocker is used at a 1.2 to 2-fold higher concentration than the concentration of forward and reverse primers. The primer complementary to the blocker preferably is modified to prevent strand displacement during amplification; in a preferred embodiment, this primer contains 2'-O-methyl at the position complementary to the 5' end of the blocker in order to prevent strand displacement.

DEPR:
Three oligodeoxynucleotides complementary to segments of human prostate specific antigen (PSA) DNA were synthesized (FIG. 12). Reverse primer contained a 2'-O-methyl moiety at a position complementary to the 5'-end of the blocker. This modification was essential for prevention of strand displacement during the amplification process (see Section 5.2.2.1) The blocker had biotin on its 3' end, in order to protect it from 3'-5' exonuclease hydrolysis and from undesirable extension during amplification. During the synthesis of blocker and forward primer, the primary amino group was incorporated on the modified T-base (Amino-Modifier C6 dT) as described by Ju et al. (1995, Proc. Natl. Acad. Sci. USA 92:4347-4351). These modifications were used for subsequent incorporation of fluorescent dyes into designated positions of the oligonucleotides. Synthesized oligonucleotides were desalted and FAM (as a donor) and rhodamine (as an acceptor) were attached to a modified thymidine residue of the reverse primer and blocker, respectively, by the method published by Ju et al. (1995, Proc. Natl. Acad. Sci. USA 92:4347-4351). Labeled oligonucleotides were purified on a 15% denaturing polyacrylamide gel.

Full | Title | Citation | Front | Review | Classification | Date | Reference   KWIC | Draw Desc | Image

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☐ 7. Document ID: US 6027889 A
L1: Entry 7 of 15 File: USPT Feb 22, 2000

DOCUMENT-IDENTIFIER: US 6027889 A
TITLE: Detection of nucleic acid sequence differences using coupled ligase detection and polymerase chain reactions

DEPR:
FIG. 12 is a schematic drawing showing a solution to the allele specific LDR/PCR problem, utilizing an intermediate exonuclease digestion step. Allele-specific LDR/PCR can be achieved while significantly reducing background ligation independent (incorrect) target amplification. To do so, it is necessary to eliminate one or more of the components required for ligation independent PCR amplification, without removing the information content of the ligation product sequence. One solution is to use exonuclease in step 2 to digest unreacted LDR oligonucleotide probes from step 1. By blocking the end which is not ligated, for example the 3' end of the downstream oligonucleotide probe, one probe can be made substantially resistant to digestion, while the other is sensitive. Only the presence of full length ligation product sequence will prevent digestion of the upstream primer. Blocking groups include use of a thiophosphate group and/or use of 2-O-methyl ribose sugar groups in the backbone. Exonucleases include Exo I (3'-5'), Exo III (3'-5'), and Exo IV (both 5'-3' and 3'-5'), the later requiring blocking on both sides. One convenient way to block both probes is by using one long "padlock" probe (see M. Nilsson et. al., "Padlock Probes: Circularizing Oligonucleotides for Localized DNA Detection," Science 265: 2085-88 (1994), which is hereby incorporated by reference), although this is by no means required. An advantage of using exonucleases, for example a combination of Exo I (single strand specific) and Exo III (double strand specific), is the ability to destroy both target and one LDR probe, while leaving the ligation product sequences substantially undigested. By using an exonuclease treatment prior to PCR, in accordance with steps 3 and 4, either one or both oligonucleotide probes in each set are substantially reduced, and thus hybridization of the remaining oligonucleotide probes to the original target DNA (which is also substantially reduced by exonuclease treatment) and formation of a ligation product sequence which is a suitable substrate for PCR amplification by the oligonucleotide primer set is substantially reduced. In other words, formation of ligation independent labeled extension products is substantially reduced or eliminated.

Full | Title | Citation | Front | Review | Classification | Date | Reference

KWIC | Draw Desc | Image

8. Document ID: US 6013510 A
L1: Entry 8 of 15 File: USPT Jan 11, 2000

DOCUMENT-IDENTIFIER: US 6013510 A
TITLE: Identification of a DNA region potentially useful for the detection of Mycobacterium kansasii

DEPR:
Suitable bases for preparing the oligonucleotide probes or amplification primers of the present invention may be selected from naturally occurring nucleotide bases such as adenine, cytosine, guanine, uracil, and thymine; and non-naturally occurring or "synthetic" nucleotide bases such as 8-oxo-guanine, 6-mercaptoguanine, 4-acetylcytidine, 5-(carboxyhydroxyethyl)uridine, 2'-O-methylcytidine, 5-carboxymethylamino-methyl-2-thioridine, 5-carboxymethylaminomethyluridine, dihydrouridine, 2'-O-methylpseudouridine, .beta.,D-galactosylqueosine, 2'-O-methylguanosine, inosine, N6-isopentenyladenosine, 1-methyladenosine, 1-methylpseudouridine, 1-methylguanosine, 1-methylinosine, 2,2-dimethylguanosine, 2-methyladenosine, 2-methylguanosine, 3-methylcytidine, 5-methylcytidine, N6-methyladenosine, 7-methylguanosine, 5-methylaminomethyluridine, 5-methoxyaminomethyl-2-thiouridine, .beta.,D-mannosylqueosine, 5-methoxycarbonylmethyluridine, 5-methoxyuridine, 2-methylthio-N6-isopentenyladenosine, N-((9-.beta.-D-ribofuranosyl-2-methylthiopurine-6-yl)carbamoyl)threonine, N-((9-.beta.-D-ribofuranosylpurine-6-yl)N-methylcarbamoyl)threonine, uridine-5-oxyacetic acid methylester, uridine-5-oxyacetic acid, wybutoxosine, pseudouridine, queosine, 2-thiocytidine, 5-methyl-2-thiouridine, 2-thiouridine, 2-thiouridine, 5-methylurdine, N-((9-.beta.-D-ribofuranosylpurine-6-yl)carbamoyl)threonine, 2'-O-methyl-5-methyluridine, 2'-O-methylurdine, wybutosine, and 3-(3-amino-3-carboxypropyl)uridine. Any oligonucleotide backbone may be employed, including DNA, RNA (although RNA is less preferred than DNA), modified sugars such as carbocycles, and sugars containing 2' substitutions such as fluoro and methoxy. The oligonucleotides may be oligonucleotides wherein at least one, or all, of the internucleotide bridging phosphate residues are modified phosphates, such as methyl phosphonates, methyl phosphonothioates, phosphoromorpholidates, phosphoropiperazidates and phosphoramidates (for example, every other one of the internucleotide bridging phosphate residues may be modified as described). The oligonucleotide may be a "peptide nucleic acid" such as described in P. Nielsen et al., Science 254, 1497-1500 (1991). The only requirement is that the oligonucleotide probe should possess a sequence at least a portion of which is capable of binding to a portion of the sequence of a target DNA molecule.

Full | Title | Citation | Front | Review | Classification | Date | Reference | KWIC | Draw Desc | Image

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9. Document ID: US 6004754 A

L1: Entry 9 of 15 File: USPT Dec 21, 1999

DOCUMENT-IDENTIFIER: US 6004754 A
TITLE: DNA sequence, related probes and primers for the detection of Streptococcus agalactiae

DEPR:
Suitable bases for preparing the oligonucleotide probes or amplification primers of the present invention may be selected from naturally occurring nucleotide bases such as adenine, cytosine, guanine, uracil, and thymine; and non-naturally occurring or "synthetic" nucleotide bases such as 8-oxo-guanine, 6-mercaptoguanine, 4-acetylcytidine, 5-(carboxyhydroxyethyl)uridine, 2'-O-methylcytidine, 5-carboxymethylamino-methyl-2-thioridine, 5-carboxymethylaminomethyluridine, dihydrouridine, 2'-O-methylpseudouridine, .beta.,D-galactosylqueosine, 2'-O-methylguanosine, inosine, N.sup.6 -isopentenyladenosine, 1-methyladenosine, 1-methylpseudouridine, 1-methylguanosine, 1-methylinosine, 2,2-dimethylguanosine, 2-methyladenosine, 2-methylguanosine, 3-methylcytidine, 5-methylcytidine, N.sup.6 -methyladenosine, 7-methylguanosine, 5-methylaminomethyluridine, 5-methoxyaminomethyl-2-thiouridine, .beta.,D-mannosylqueosine, 5-methoxycarbonylmethyluridine, 5-methoxyuridine, 2-methylthio-N.sup.6 -isopentenyladenosine, N-((9-.beta.-D-ribofuranosyl-2-methylthiopurine-6-yl)carbamoyl)threonine, N-((9-.beta.-D-ribofuranosylpurine-6-yl)N-methylcarbamoyl)threonine, uridine-5-oxyacetic acid methylester, uridine-5-oxyacetic acid, wybutoxosine, pseudouridine, queosine, 2-thiocytidine, 5-methyl-2-thiouridine, 2-thiouridine, 2-thiouridine, 5-methyluridine, N-((9-.beta.-D-ribofuranosylpurine-6-yl)carbamoyl)threonine, 2'-O-methyl-5-methyluridine, 2'-O-methyluridine, wybutosine, and 3-(3-amino-3-carboxypropyl)uridine.

Full | Title | Citation | Front | Review | Classification | Date | Reference   KWIC | Draw Desc | Image

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☐ 10. Document ID: US 5985569 A
L1: Entry 10 of 15   File: USPT   Nov 16, 1999

DOCUMENT-IDENTIFIER: US 5985569 A
TITLE: Primers for amplification of a genus specific sequence of the mycobacterium 16S rRNA gene

DEPR:
Suitable bases for preparing the oligonucleotide probes or amplification primers of the present invention may be selected from naturally occurring nucleotide bases such as adenine, cytosine, guanine, uracil, and thymine; and non-naturally occurring or "synthetic" nucleotide bases such as 8-oxo-guanine, 6-mercaptoguanine, 4-acetylcytidine, 5-(carboxyhydroxyethyl)uridine, 2'-O-methylcytidine, 5-carboxymethylamino-methyl-2-thioridine, 5-carboxymethylaminomethyluridine, dihydrouridine, 2'-O-methylpseudouridine, .crclbar.,D-galactosylqueosine, 2'-O-methylguanosine, inosine, N6-isopentenyladenosine, 1-methyladenosine, 1-methylpseudouridine, 1-methylguanosine, 1-methylinosine, 2,2-dimethylguanosine, 2-methyladenosine, 2-methylguanosine, 3-methylcytidine, 5-methylcytidine, N6-methyladenosine, 7-methylguanosine, 5-methylaminomethyluridine, 5-methoxyaminomethyl-2-thiouridine, .crclbar.,D-mannosylqueosine, 5-methoxycarbonylmethyluridine, 5-methoxyuridine, 2-methylthio-N6-isopentenyladenosine, N-((9-.crclbar.-D-ribofuranosyl-2-methylthiopurine-6-yl)carbamoyl)threonin e, N-((9-.crclbar.-D-ribofuranosylpurine-6-yl)N-methyl-carbamoyl)threonine, uridine-5-oxyacetic acid methylester, uridine-5-oxyacetic acid, wybutoxosine, pseudouridine, queosine, 2-thiocytidine, 5-methyl-2-thiouridine, 2-thiouridine, 2-thiouridine, 5-methyluridine, N-((9.crclbar.-D-ribofuranosylpurine-6-yl)carbamoyl)threonine, 2'-O-methyl-5-methyluridine, 2'-O-methylurdine, wybutosine, and 3-(3-amino-3-carboxypropyl)uridine. Any oligonucleotide backbone may be employed, including DNA, RNA (although RNA is less preferred than DNA), modified sugars such as carbocycles, and sugars containing 2' substitutions such as fluoro and methoxy. The oligonucleotides may be oligonucleotides wherein at least one, or all, of the internucleotide bridging phosphate residues are modified phosphates, such as methyl phosphonates, methyl phosphonothioates, phosphoromorpholidates, phosphoropiperazidates and phosphoramidates (for example, every other one of the internucleotide bridging phosphate residues may be modified as described). The oligonucleotide may be a "peptide nucleic acid" such as described in P. Nielsen et al., Science 254, 1497-1500 (1991). The only requirement is that the oligonucleotide probe should possess a sequence at least a portion of which is capable of binding to a portion of the sequence of a target DNA molecule.

Full | Title | Citation | Front | Review | Classification | Date | Reference | KWIC | Draw Desc | Image

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☐ 11. Document ID: US 5952202 A

L1: Entry 11 of 15 File: USPT Sep 14, 1999

DOCUMENT-IDENTIFIER: US 5952202 A
TITLE: Methods using exogenous, internal controls and analogue blocks during nucleic acid amplification

BSPR:
The block may be comprised of modifications to the internucleotide linkage, the sugar, or nucleobase moieties of a DNA primer to render it non-extendable by polymerase. An example of a suitable modification is a 3' phosphate group. Analogs of DNA may be employed as the block, such as, 2-aminoethylglycine, peptide-nucleic acid (PNA) and other amide-linked oligomers; 2'-O-methyl and other 2'-O-alkyl oligoribonucleotides; phosphorothioate and other phosphate analogs; and the like. The block is selected for several properties, including (i) high specificity, (ii) high affinity, (iii) non-extendability, (iv) chemical stability, (v) non-interference with amplification. In a preferred embodiment, the block is a PNA (peptide-nucleic acid) oligomer (Nielsen, P. E. etal). To improve solubility and lower aggregation effects, the PNA block may be conjugated with hydrophilic labels, such as polyethyleneoxy, peptides, nucleic acids, nucleic acid analogs, and the like.

Full | Title | Citation | Front | Review | Classification | Date | Reference | KWIC | Draw Desc | Image

12. Document ID: US 5942609 A

L1: Entry 12 of 15 File: USPT Aug 24, 1999

DOCUMENT-IDENTIFIER: US 5942609 A
TITLE: Ligation assembly and detection of polynucleotides on solid-support

DEPR:
Nucleotide 5' triphosphates (NTP) suitable for use in the extension step of the synthesis methods of the invention or for use in the amplification of the polynucleotide by polymerase chain reaction include any that are capable of being polymerized by a polymerase enzyme. Suitable NTPs include both naturally occurring and synthetic nucleotide triphosphates, and are not limited to, ATP, dATP, CTP, dCTP, GTP, dGTP, UTP, TTP, dUTP, 5-methyl-CTP, 5-methyl-dCTP, ITP, dITP, 2-amino-ATP, 2-amino-dATP, as well as the .alpha.-thiotriphosphates, 2'-O-methyl-ribonucleotide 5'-triphosphates 2'-fluoro-NTP, and 2'-amino-NTP for all of the above. Preferably, the nucleotide triphosphates used in the methods of invention are selected from the group consisting of dATP, dCTP, dGTP, TTP, and mixtures thereof Modified nucleobases can also be used, including but not limited to, 5-Br-UTP, 5-Br-dUTP, 5-F-TTP, 5-F-dUTP, 5-propynyl dCTP, and 5-propynyl-dUTP. Most of these nucleotide triphosphates are widely available from commercial sources such as Sigma Chemical Co., St. Louis, Mo. Nucleotide triphosphates are advantageously used in the methods of the present invention at least because they are generally cheaper than the phosphoramidite nucleoside monomers used in the chemical synthesis of oligonucleotides.

Full | Title | Citation | Front | Review | Classification | Date | Reference | KWIC | Draw Desc | Image

13. Document ID: US 5866336 A

L1: Entry 13 of 15 File: USPT Feb 2, 1999

DOCUMENT-IDENTIFIER: US 5866336 A
TITLE: Nucleic acid amplification oligonucleotides with molecular energy transfer labels and methods based thereon

DEPR:
A schematic illustration of gap triamplification, which consists of repeated elongation and ligation of the amplification product, is shown in FIG. 7. Blocker may be used at the same or higher concentration than the concentration of forward and reverse primers. Preferably, blocker is used at a 1.2 to 2-fold higher concentration than the concentration of forward and reverse primers. The primer complementary to the blocker preferably is modified to prevent strand displacement during amplification; in a preferred embodiment, this primer contains 2'-O-methyl at the position complementary to the 5' end of the blocker in order to prevent strand displacement.

DEPR:
Three oligodeoxynucleotides complementary to segments of human prostate specific antigen (PSA) DNA were synthesized (FIG. 12). Reverse primer contained a 2'-O-methyl moiety at a position complementary to the 5'-end of the blocker. This modification was essential for prevention of strand displacement during the amplification process (see Section 5.2.2.1) The blocker had biotin on its 3' end, in order to protect it from 3'-5' exonuclease hydrolysis and from undesirable extension during amplification. During the synthesis of blocker and forward primer, the primary amino group was incorporated on the modified T-base (Amino-Modifier C6 dT) as described by Ju et al. (1995, Proc. Natl. Acad. Sci. USA 92:4347-4351). These modifications were used for subsequent incorporation of fluorescent dyes into designated positions of the oligonucleotides. Synthesized oligonucleotides were desalted and FAM (as a donor) and rhodiamine (as an acceptor) were attached to a modified thymidine residue of the reverse primer and blocker, respectively, by the method published by Ju et al. (1995, Proc. Natl. Acad. Sci. USA 92:4347-4351). Labeled oligonucleotides were purified on a 15% denaturing polyacrylamide gel.

Full | Title | Citation | Front | Review | Classification | Date | Reference   KWIC | Draw Desc | Image

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☐ 14. Document ID: US 5747252 A
L1: Entry 14 of 15   File: USPT   May 5, 1998

DOCUMENT-IDENTIFIER: US 5747252 A
TITLE: Nucleic acid probes and amplification oligonucleotides for Neisseria species

BSPR:
By "oligonucleotide" is meant a single-stranded nucleotide polymer made of more than 2 nucleotide subunits covalently joined together. Preferably between 10 and 100 nucleotide units are present, most preferably between 12 and 50 nucleotides units are joined together. The sugar groups of the nucleotide subunits may be ribose, deoxyribose or modified derivatives thereof such as 2'-O-methyl ribose. The nucleotide subunits of an oligonucleotide may be joined by phosphodiester linkages, phosphorothioate linkages, methyl phosphonate linkages or by other rare or non-naturally-occurring linkages that do not prevent hybridization of the oligonucleotide. Furthermore, an oligonucleotide may have uncommon nucleotides or non-nucleotide moieties. An oligonucleotide as defined herein is a nucleic acid, preferably DNA, but may be RNA or have a combination of ribo- and deoxyribonucleotides covalently linked. Oligonucleotide probes and amplification oligonucleotides of a defined sequence may be produced by techniques known to those of ordinary skill in the art, such as by chemical or biochemical synthesis, and by in vitro or in vivo expression from recombinant nucleic acid molecules, e.g., bacterial or retroviral vectors. As intended by this disclosure, an oligonucleotide does not consist of wild-type chromosomal DNA or the in vivo transcription products thereof. One use of a probe is as a hybridization assay probe; probes may also be used as in vivo or in vitro therapeutic amplification oligomers or antisense agents to block or inhibit gene transcription, or translation in diseased, infected, or pathogenic cells.

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15. Document ID: US 5679553 A

L1: Entry 15 of 15   File: USPT   Oct 21, 1997

DOCUMENT-IDENTIFIER: US 5679553 A
TITLE: Process for rendering a nucleic acid amplication reaction product incapable of being a target for further amplification, a diagnostic assay employing said process

BSPR:
In the process according to the invention the detection oligonucleotide preferably comprises a 3' terminus that cannot be elongated with another nucleotide. The means by which this can be achieved is known to a person skilled in the art. Furthermore, in a suitable embodiment of the invention the step of partial degradation of the hybridisation complex can be executed by using a nuclease for which the complex of amplified product and detection oligonucleotide is insusceptible to degradation. An example of a detection oligonucleotide being insusceptible to degradation by nuclease is an oligonucleotide comprising 2'-O -methyl modification of a number of nucleotides sufficient to prevent degradation by nuclease, preferably comprising only nucleotides modified in said manner. In the case of a NASBA amplification reaction both DNA and RNA have to be degraded simultaneously in order for the method to be successful as both DNA and RNA are amplified. Therefore, in such a case the process according to the invention can be carried out with a nuclease or a mixture of nucleases directed at degrading both DNA and RNA. It is also possible to carry out the degradation using specific DNase such as DNase I, a restriction enzyme, an endonuclease or an exonuclease for solely degrading DNA. Specifically for degrading RNA, specific RNase such as RNase T can be used, as can RNase U, RNase A or an exonuclease. It is also possible to carry out the degradation using non- specific nuclease capable of simultaneous degradation of both DNA and RNA. In another embodiment the degradation step of the process can be carried out chemically rather than enzymstically with nuclease.

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In a suitable embodiment a kit according to the invention comprises an oligonucleotide with a modification in the form of 2'-O-methyl on a sufficient number of nucleotides to render the hybridized part of the complex of amplified product and oligonucleotide undegradable by nuclease, said modiifcation preferably being present on all nucleotides of the oligonucleotide. Furthermore, a kit especially suited for carrying out the invention will preferably further comprise the compound or compounds required for partical degradation of the nucleic acid, e.g. the nuclease in sufficient amount for degradation of the amplified nucleic acid. The specific nuclease or nucleases to be included will depend on the amplification reaction to be carried out, e.g. DNase and RNase for NASBA or DNase for PCR. With a view to minimizing the number of times the vessel comprising amplified product is opened the kit according to the invention should comprise the compound or compounds required for partial degradation of the nucleic acid, e.g. the nuclease in a container in which the process is to be carried out in such a manner that the compound or compounds required for partial degradation of the nucleic acid, e.g. the nuclease can only contact the reaction mixture after hybridisation of the oligonucleotide or oligonucleotides to the amplified product has occurred. A container suitable for carrying out the process according to the invention or for use in a kit according to the invention falls within the scope of the invention. Such a container comprises the compound or compounds required for partial degradation of the nucleic acid, e.g. the nuclease in a separate compartment in the container, said separate compartment being degradable enabling contact of the compound or compounds required for partial degradation of the nucleic acid, e.g. the nuclease with the rest of the container after degradation of the separate compartment. The wall of the separate compartment can, for example, comprise a thermodegradable substance, degradable at a temperature below the inactivation temperature of the compound or compounds required for partial degradation of the nucleic acid, e.g. the nuclease and below the melting out temperature of the hybridisation complex. Examples of such a thermodegradable substance are wax or agarose. Any other sort of equivalent delayed release system of the compound or compounds required for partial degradation of the nucleic acid, e.g. the nuclease will be obvious to a person skilled in the art. The idea being that the container in which the reactio or amplification is carried out is opened as little as possible in order to reduce the risk of carry-over. The invention is further illustrated in the following Examples, and should not be considered as being only restricted thereto.

DEPR:
NASBA amplifications and nuclease treatments are as described in Example 1. Following the nuclease treatment, or coinciding with the nuclease treatment the NASBA amplified product should be detected. Detection of the NASBA amplified product should be sequence specific, i.e. using one or more specific oligonucleotide probes. The following experiment was performed, after NASBA amplification the amplified products (1 .mu.l) were hybridized with an oligonucleotide (10.sup.12 molecules) in 1.times.NRG buffer for 30 minutes at 45.degree. C. Hybridisations were done with single standard oligonucleotides, single 2'-O-methyl oligonucleotides, two adjacent standard oligonucleotides and two adjacent 2'-O-methyl oligonucleotides. The hybridisations were followed by RNase A (50 mg) and DNase I (500 units) treatment (see example 1). The nuclease treated hybdridisation examples were analysed on 20% acrylamide gel stained with ethidium bromide. The results are summarized in Table 5.

DEPR:
NASBA amplification and nuclease treatment are the same as described in Example 1. The NASBA amplified products were subject to hybridisation with 2'-O-methyl oligonucleotides using a sandwich format with magnetic beads, a biotinylated capture probe and a HRP-labelled detection probe. For this hybridisation both a one-step and a two-step protocol were used.

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